We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 3

Angiogenesis Meets Skeletogenesis: The Cross-Talk between Two Dynamic Systems

Tamara A. Franz-Odendaal, Daniel Andrews and Shruti Kumar

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66497

Abstract

In this chapter, we describe the complex relationship between angiogenesis and skeletogenesis. While much is known about the interactions of these two dynamic systems for bones that ossify via a cartilage template, comparatively little is known about directly ossifying bones. Most of the bones of the head develop from osteogenic condensations and undergo intramembranous (direct) ossification during development. Our understanding of the relationship between osteogenic cell condensations (in particular) and angiogenesis is currently inadequate and prevents a comprehensive understanding of vertebrate head development. This chapter highlights our understanding of both direct and indirectly ossifying bones shedding light on where there are important gaps in our understanding.

Keywords: avascular, skeletogenic condensation, osteoblasts, chondrocytes, cell culture, VEGF, HIF

1. Introduction

The coordinated development of tissues is critical for proper development. Bone is a highly vascularized tissue that also houses the hematopoetic cell niche, which provides a lifelong supply of blood cells (in humans and most other vertebrates). In this chapter, we will explore the role angiogenesis plays during the development of bones. Bones that ossify endochondrally (from cartilage) versus bones that ossify intramembranously (without cartilage) have different relationships with vasculature and hence with the process of angiogenesis. These bones start development by forming a skeletogenic condensation, however, the molecular signals within these condensations differ [1]. We begin with a discussion of bones that

develop from a cartilage precursor (endochondral ossification) since more is known about this process than about directly ossifying bones (intramembranous ossification). We also discuss data from cell culture and bone grafts that shed light on the cross talk between these two dynamic processes, angiogenesis and skeletogenesis. Some pathological implications are also included.

2. Bones that ossify via endochondral ossification

2.1. Angiogenesis during formation of the cartilage template

Endochondrally ossifying bones are bones that form via a cartilage template. The process of endochondral ossification begins with stem cells originating from the mesenchyme in the future area of bone development. These stem cells aggregate to form a chondrogenic condensation. Once this cell aggregation reaches a critical size, cells begin to differentiate into chondrocytes (chondroblasts) that will then secrete extracellular matrix (ECM), collagen type II. This matrix matures into the cartilage template that will eventually ossify (e.g., long bones). Cartilage itself is avascular when first deposited, however, differentiated chondrocytes secrete anti-angiogenic factors (inhibitors) to maintain the avascular nature of the cartilage. These early chondrocytes are typically referred to as pre-hypertrophic chondrocytes. As these chondrocytes mature, they gradually become hypertrophic and begin to secrete a number of pro-angiogenic factors. Vascular endothelial-derived growth factor (VEGF), a protein which is important for angiogenesis and the subsequent ossification of the cartilage, is a key angiogenic factor during bone development. Hypoxia-inducible factor is one of the key upstream regulators of VEGF, and changes in the level of HIF can alter the level of VEGF thus dramatically changing bone mass (discussed later).

During the maturation of hypertrophic chondrocytes, these cells release VEGF into the extracellular matrix and into the area surrounding the perichondrium layer of the cartilage. The hypertrophic chondrocytes also secrete fibroblast growth factor (FGF), bone morphogenetic protein (BMP) and connective tissue growth factor (CTGF) [2, 3]. With the arrival of these factors to the site of bone formation, angiogenesis in the bone can now occur. There is a complex cross talk between bone-forming cells (osteoblasts), the angiogenic factors present, and the invading vasculature. These growth factors, in particular VEGF, recruit osteo/chondro-clasts, osteoprogenitors and other bone precursors to the area in order to initiate the primary ossification center, the first site of bone formation within the cartilage template [2, 4].

Osteoclasts are bone matrix resorbing cells, and once recruited, they secrete multiple factors that contribute to the initiation of vascularization, including the release of more VEGF and FGFs [2, 5]. Among these factors are matrix metalloproteinases (MMPs) and hypoxia-inducible factors (HIFs) which, along with VEGF, are other key proteins important in the development of vasculature in bone. The MMPs break down the ECM and release matrix-bound VEGF that allows for the resorption of cartilage so that osteoblasts can infiltrate and deposit bone. MMPs, primarily MMP9 and MMP13, also function to recruit osteoclasts and osteoprogenitors, further aiding in the subsequent deposition of bone [2, 4]. The matrix-bound VEGF that is released is a key player in the formation of bone vasculature. HIFs regulate VEGF

http://dx.doi.org/10.5772/66497

expression in bone, which has an effect on angiogenesis [2]. Thus VEGF, MMPs and HIFs play a central role in angiogenesis during endochondral ossification.

2.2. Vascular invasion of the cartilage

Vascular invasion of the cartilage template is the first major step in angiogenesis in the developing bone. The vasculature from the perichondrium penetrates the previously avascular cartilage and begins to invade the rest of the cartilage. Vascular invasion is coupled to a number of events in the cartilage, the degradation of the extracellular matrix by MMPs, which releases matrix-bound VEGF and favors the invasion of the cartilage, as well as apoptosis of the hypertrophic chondrocytes which were responsible for secreting many of the growth factors that resulted in the invasion [2, 4, 6]. As vasculature penetrates the cartilage and the hypertrophic chondrocytes undergo apoptosis, they are promptly replaced by osteoblasts in the resulting bone cavity (i.e., by the osteoblasts previously recruited by factors such as VEGF). These osteoblasts then secrete collagen type I to initiate ossification to form the bone matrix in the primary ossification center. The same growth factors in and around the growth plate of the bone will allow the vasculature to continue its invasion of the bone to the secondary ossification centers located at either ends of the long bone, in the same way that the primary ossification centers were formed [2].

Angiogenesis during endochondral ossification is heavily dependent on the cellular components of the skeleton, namely osteoblasts and chondrocytes. Without these cells, the valuable pro-angiogenic factors would not be properly secreted and will not be able to induce vascular invasion. However, the reverse is also true. In order for bone to continue to form properly, efficient and effective vascular invasion is required. The vasculature that invades cartilage likely also carries required factors for bone growth; the vascular epithelium is thought to carry osteoprogenitors, more VEGF, FGFs and other factors which assist with the resorption of the surrounding cartilage and the ossification of bone [6]. Without vasculature invading the cartilage, bone formation is impaired. A study on mouse long bones showed that expression of SOX9 can block the expression of VEGFA, which results in impaired vascular invasion, and as a result, the bones do not ossify [7]. (SOX9 is a key marker of chondrogenic condensations [1].) VEGF also plays a role in the ongoing maintenance of bone vasculature endogenous VEGF was shown to enhance vascularization of bone and subsequently allows more rapid healing of injured bones, indicating the role of vascularization in the formation and turnover of endochondral bone [6].

Along with VEGF, another key factor in the invasion of vasculature into bone is hypoxia. The lack of oxygen will result in the release of hypoxia-inducible factors (HIFs) which regulate the production of the extracellular matrix and VEGF expression. The matrix binds VEGF triggering vascular invasion. The HIFs expressed by osteoblasts support both the proper vascularization of bone as well as the proper functioning of osteoblasts. Increased expression of HIFs results in more vascularization and thicker bones, whereas deletion of HIFs (and the resulting reduced expression of VEGF) results in less vascularization and thinner bones [2]. Thus, VEGFs and HIFs are two major factors that couple angiogenesis to endochondral bone formation [2, 6].

Thus in summary, endochondral ossification is dependent on the interaction between pro-angiogenic factors acting on bone-forming cells (**Figure 1**), and endochondral ossification is impaired in the absence of these factors.

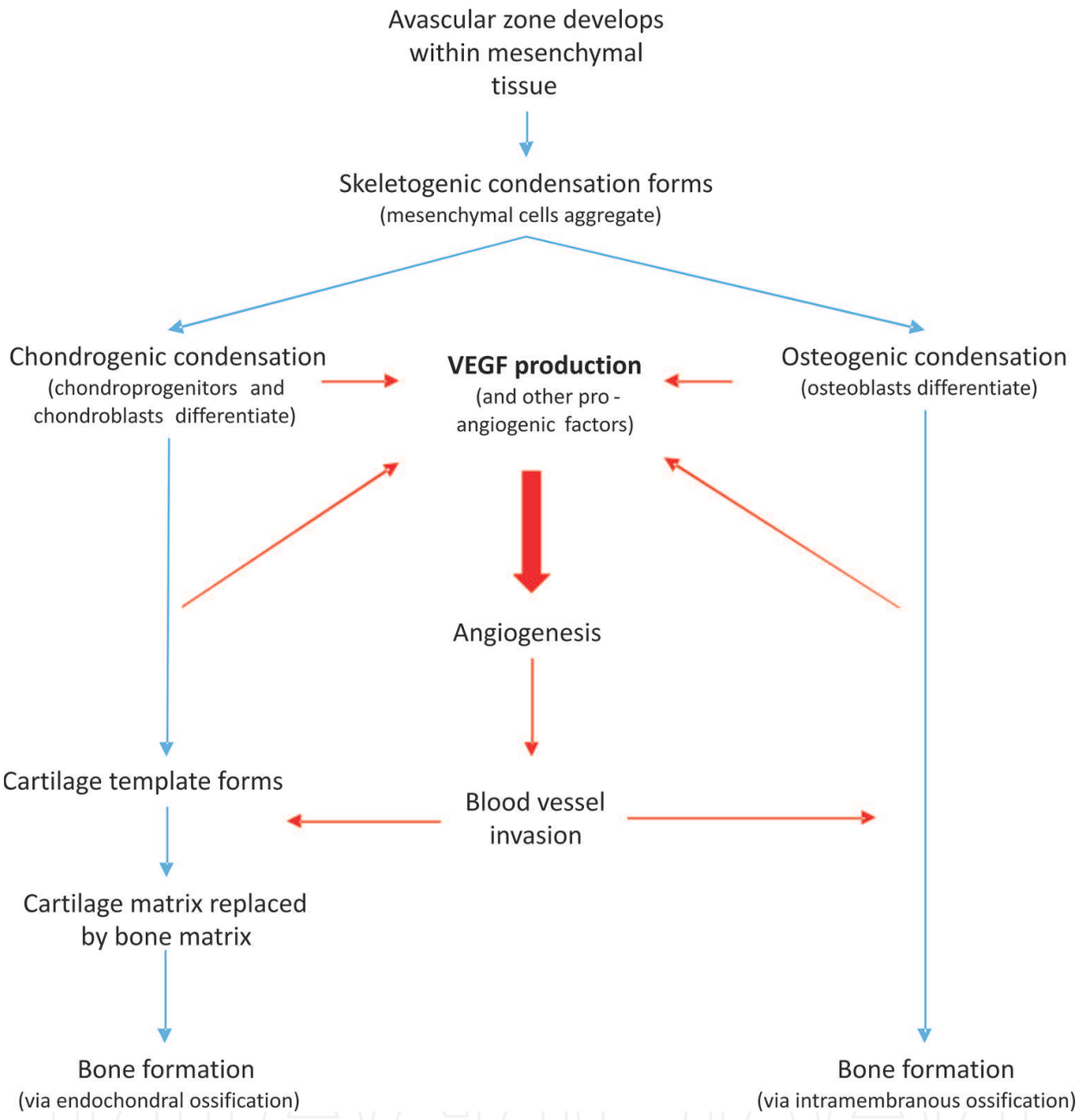

Figure 1. Schematic showing the complex cross talk between angiogenesis and skeletogenesis. Mesenchymal cells aggregate in an avascular (hypoxic) zone to form skeletogenic condensations. Differences in the molecular characteristics of these cells determine the fate of the condensation. Cells within this condensation produce VEGF and other pro-angiogenic factors, and this induces angiogenesis. Left side: Cells within the chondrogenic condensation continue to produce these angiogenic factors, and ultimately, the condensation differentiates into a cartilage template, which is still avascular. Following angiogenesis surrounding this template, blood vessels invade the cartilage perichondrium layer, and this triggers osteoblast differentiation, cartilage matrix degradation, bone matrix production and the further release of more pro-angiogenic factors. Ossification begins in the diaphysis (shaft) of long bones and spreads to the epiphyses (ends of the bone). Right side: As cells within the skeletogenic condensation differentiate, they continue to produce angiogenic factors. These factors induce angiogenesis and subsequent blood vessel invasion into the outer edges of the condensation. As more cells within the condensation differentiate, the wave of bone matrix deposition and blood vessel invasion spreads outwards.

http://dx.doi.org/10.5772/66497

3. Bones that ossify via intramembranous ossification

3.1. Directly ossifying bones

Intramembranously ossifying bones form without a cartilage template. Motile mesenchymal cells fated to differentiate into osteoblasts aggregate to from skeletogenic condensations at the site of the future bone. As these osteogenic aggregations enlarge and reach a critical size, the central cells begin to differentiate into osteoblasts, lose their mobility and deposit bone matrix. This process results in osteoblasts becoming embedded or trapped in bone matrix, forcing them to differentiate into osteocytes [8]. The majority of the craniofacial skeleton forms via intramembranous ossification [9]. A common example is the skull vault (the calvariae). Less common examples are the scleral ossicles (in reptiles), parts of the clavicles and scapula (in mammals), the cleithra and opercula (in fish) and sesamoid bones (e.g., the patella in humans) [9]. Although vascularization has been extensively studied in endochondral ossification as discussed above, comparatively little research has been conducted to understand the relationship between angiogenesis and intramembranous ossification.

3.2. Angiogenesis during formation of the initial phase of directly ossifying bones

The most studied intramembranous bones are the calvariae (or skull vault). Cells in the osteogenic condensations proliferate resulting in growth of the condensations until a critical size is reached. Ossification begins at the center of the condensation and expanding outwards toward the apex of the head [10]. Once this has occurred, cells at the osteogenic front proliferate, and the bones grow toward one another [11]. Areas that ossified first form a trabecular bone structure that later becomes woven bone [10]. Interestingly, the frontal and parietal bones in humans each develop two condensations, each with their own ossification centers; these centers then fuse as ossification progresses [12].

There is a significant difference in the gene expression patterns in prechondrogenic and preosteogenic condensations [1]. Avascularity within condensations may be necessary for the formation of the condensations themselves and/or to provide positional cues [10, 13]. Indeed, in scleral ossicles, an avascular zone develops surrounding the condensation [14, 15]. Manipulating the size of this avascular zone has a direct effect on subsequent bone development [15]. Although not much is known about the process of vascularization during intramembranous ossification, it is thought that similar to endochondral bones, hypoxic conditions are important to induce angiogenesis. Avascular zones likely surround all preosteogenic condensations in mammals and avians, however, the mechanism by which these zones are established is not known [10]. Percival and colleagues [10] postulate that this avascularity may be important for condensation growth, and subsequent intramembranous ossification (as in the case for prechondrogenic condensations of endochondrally ossifying bones, **Figure 1**).

A single study describes in detail, the association of angiogenesis and intramembranous bones [16]. This study of the development of the chick frontal bone showed that small capillaries invade the thin avascular layer of loose mesenchymal cells of the condensation prior to

invading the condensation at/near the site of initial ossification [16]. This association between vascular invasion and ossification continues and cascades as the bone expands in all directions. As the bone mineralizes in the wake of this vascular invasion front, the internal and external vasculature is remodeled.

Based on studies of endochondral ossification and distraction osteogenesis, Percival and colleagues [10] recently developed a model of angiogenesis during intramembranous ossification. They propose that prior to the onset of mineralization, small capillaries begin to invade the surrounding avascular loose mesenchymal tissue due to the presence of pro-angiogenic factors. At around the time of mineralization onset, these capillaries invade the osteogenic condensations and branch outwards from the ossification center (**Figure 1**). These capillaries branch toward regions of pro-angiogenic factor expression and thus support the proliferating mesenchymal cells of the condensation. Mineralization thus first occurs at sites closest to the capillaries and then at sites progressively further away. Once the osteogenic condensation stops expanding, the capillaries along with the mineralization front continue to moves toward the presumptive sutures (i.e., the edges of the future bone), thus allowing continued calvarial growth.

Interestingly, while osteoblasts in endochondrally ossifying bones express both HIF1α and HIF2α, only HIF2α is detected in the osteoblasts of directly ossifying bones (i.e., those that undergo intramembranous ossification) [17]. This altered expression pattern of the HIFα subunits could suggest that alternative regulatory pathways trigger angiogenesis in these distinct types of ossification [17]. Percival and Richtsmeier [10] provide a comprehensive list of hypotheses relating to intramembranous osteogenesis and angiogenesis that require testing. The cross talk between these two dynamic processes is summarized in **Figure 1**.

4. Insights from cell culture and bone graft studies

VEGF is a chemoattractant for primary osteoblasts and mesenchymal progenitor cells [18] and can directly promote differentiation of primary human osteoblasts in culture in a dose-dependent manner [19]. Osteoblasts and mesenchymal stem cells are the two cell types most often used in bone tissue engineering. Interestingly, the type of cell used can influence the mode of ossification that occurs and the organization of blood vessels [20]. Implantation of osteoblasts leads to the formation of fibrous tissue and disorganized blood vessels. The osteoblasts become trapped in the secreted bone matrix (i.e., intramembranous ossification occurs). In contrast, implantation of stem cells leads to the formation of cartilaginous tissue (i.e., endochondral ossification) and well-organized blood vessels.

Basic fibroblast growth factor (bFGF) is another important pro-angiogenic factor. When bone mesenchymal stem cells were transfected with bFGF and then implanted into rats with calvarial defects, an increase in vascularization and osteogenesis was observed [21]. Similarly, the addition of sonic hedgehog (Shh) in engineered bone grafts *in vitro* also promotes vascularization of the grafts [22]. Shh is expressed during fracture healing and

http://dx.doi.org/10.5772/66497

neovascularization after trauma and has been used *in vitro* to promote the organization of blood vessels in artificial tissue grafts similarly to VEGF [22, 23]. Furthermore, when these grafts were implanted subcutaneously in mice, there was increased bone formation of both directly and indirectly ossifying bone types [22]. For large defects, supplementing the graft with platelet-rich plasma results in increased bone formation [24].

5. Pathological implications

The role of growth factors like VEGF, FGF, CTGFs and others in both bone growth and angiogenesis has been demonstrated in a number of recent studies investigating the growth and healing of bones. For example, $FGF9^{+/-}$ mice exhibit a reduction in the healing of bones accompanied by a lack of VEGF expression in the area of injury, suggesting that FGF9 is required for angiogenesis and for healing long bones [25]. Hypomorphic $VEGF^{120/120}$ mice have reduced mineralization of the calvarial bones and consequently reduced bone thickness. This has been attributed to a reduction and delay in vascular invasion [26]. Additionally, conditional deletion of VEGFA in mice cranial neural crest cells causes cleft palate with reduced ossification of the mandibular bone due to reduced endothelial cell proliferation and decreased angiogenesis [27]. Mice with a VEGF-deficient osteoblastic lineage exhibit age-dependent loss of bone mass and increased bone marrow fat, similar to the changes associated with osteoporosis in humans [28].

6. Conclusions and summary

VEGF mediates angiogenesis, chondrocyte differentiation, osteoblast differentiation and osteoclast recruitment [29], and thus, its role during osteogenesis is complex ([10], **Figure 1**). Yang et al. [30] provide a useful tabulation of the effects of VEGF on intramembranous and endochondral ossification. Importantly, this chapter highlights that the relationship between angiogenesis and intramembranous ossification is not well understood. For example, only one study describes the detailed relationship between these two dynamic systems [16], and a very recent study provides a model of this process [10]. This model should be examined in several directly ossifying bones in the skeleton in order to confirm whether all directly ossifying bones follow the model or whether subtle differences exist depending perhaps on the location of the bone or the origin of the bone cells. This lack of a fundamental understanding about the developmental interactions between angiogenesis and skeletogenic condensations (particularly with respect to directly ossifying bones) contributes to our inadequate understanding of skull formation [10].

It should be noted that bones that ossify from the perichondrium of a cartilage template can be considered endochondral or intramembranous (since the perichondrium is a membrane of the cartilage). An example is Meckel's cartilage. The relationship between angiogenesis and bones that develop via the perichondrium has not been studied.

Acknowledgements

The authors acknowledge funding from the Natural Sciences and Engineering Research Council of Canada. We also acknowledge M. Bhade who collated literature for this book chapter.

Author details

Tamara A. Franz-Odendaal[1,2]*, Daniel Andrews[1] and Shruti Kumar[1,2]

*Address all correspondence to: tamara.franz-odendaal@msvu.ca

1 Mount Saint Vincent University, Nova Scotia, Canada

2 Saint Mary's University, Nova Scotia, Canada

References

[1] Eames BF, Helms JA: Conserved molecular program regulating cranial and appendicular skeletogenesis. Developmental Dynamics. 2004;231:4–13.

[2] Silvaraj KK, Adams RH: Blood vessel formation and function in bone. Development. 2016;513:2706–2715. doi:10.1242/dev.136861

[3] Liu ES, Raimann A, Chae BT, Martins JS, Baccarini M, Demay MB: C-Raf promotes angiogenesis during normal growth plate maturation. Development. 2016;143:348–355. doi:10.1242/dev.127142

[4] Ortega N, Wang K, Ferrara N, Werb Z, Vu TH: Complementary interplay between matrix metalloproteinase-9, vascular endothelial growth factor and osteoclast function drives endochondral bone formation. Disease Models and Mechanisms. 2010;3:224–235. doi:10.1242/dmm004226

[5] Berendsen AD, Olsen BR: How vascular endothelial growth factor-A (VEGF) regulated differentiation of mesenchymal stem cells. Journal of Histochemistry & Cytochemistry. 2014;62:103–108. doi:10.1369/0022155413516347

[6] Maes C: Role and regulation of vascularization processes in endochondral bones. Calcified Tissue International. 2013;92:307–323. doi:10.1007/s00223-012-9689-z

[7] Hattori T, Muller C, Gebhard S, Bauer E, Pausch F, Schlund B, Bosl MR, Hess A, Surmann-Schmitt C, von der Mark H, de Crombrugghe B, von der Mark K: SOX9 is a major negative regulator of cartilage vascularization, bone marrow formation and endochondral ossification. Development. 2010;137:901–911. doi:10.1242/dev.045203

[8] Franz-Odendaal TA, Hall BK, Witten PE: Buried alive: how osteoblasts become osteocytes. Developmental Dynamics. 2006;235:176–190.

[9] Franz-Odendaal, TA: Induction and patterning of intramembranous bone. (Special Issue – Signaling Mechanisms in Development) Frontiers in Biosciences. 2011;16: 2734–2746.

[10] Percival CJ, Richtsmeier JT: Angiogenesis and intramembranous osteogenesis. Developmental Dynamics. 2013;242(8):909–22.

[11] Rice R, Rice DP, Olsen BR, Thesleff I: Progression of calvarial bone development requires Foxc1 regulation of Msx2 and Alx4. Developmental Biology. 2003;262(1):75–87.

[12] Lemier RJ: Embryology of the skull. In Craniosynostosis: Diagnosis, Evaluation and Management. Cohen MM Jr. ed. Raven Press, New York, Chapter 5; 1986.

[13] Yin M, Pacifici M: Vascular regression is required for mesenchymal condensation and chondrogenesis in the developing limb. Devlopmental Dynamics. 2001;222:5–533.

[14] Jourdeuil K, Franz-Odendaal TA: Vasculogenesis and the induction of skeletogenic condensations in the avian eye. The Anatomical Record. 2012;285:691–698.

[15] Jabalee J, Franz-Odendaal TA: Vascular endothelial growth factor signaling regulates angiogenesis and osteogenesis during the development of scleral ossicles. Developmental Biology 2015:406:52–62.

[16] Thompson TJ, Owens PD, Wilson DJ: Intramembranous osteogenesis and angiogenesis in the chick embryo. Journal of Anatomy. 1989;166:55–65.

[17] De Spiegelaere W, Cornillie P, Casteleyn C, Burvenich C, Van Den Broeck W: Detection of hypoxia inducible factors and angiogenic growth factors during foetal endochondral and intramembranous ossification. Anatomia, Histologia, Embryologia. 2010;39(4):376–384.

[18] Fiedler J, Leucht F, Waltenberger J. et al.: VEGF-A and PIGF-1 stimulate chemotactic migration of human mesenchymal progenitor cells. Biochemical and Biophysical Research Communications. 2005;334(2):561–568.

[19] Street J, Bao M, deGuzman L. et al.: Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. Proceedings of the National Academy of Sciences of the United States of America. 2002;99(15):9656–9661.

[20] Tortelli F, Tasso R, Loiacono F, Cancedda R: The development of tissue-engineered bone of different origin through endochondral and intramembranous ossification following the implantation of mesenchymal stem cells and osteoblasts in a murine model. Biomaterials. 2010;31(2):242–249.

[21] Qu D, Li J, Li Y, Gao Y, Zuo Y, Hsu Y, Hu J: Angiogenesis and osteogenesis enhanced by bFGF ex vivo gene therapy for bone tissue engineering in reconstruction of calvarial defects. Journal of Biomedical Materials Research Part A. 2011;96(3):543–551.

[22] Rivron NC, Raiss CC, Liu J, Nandakumar A, Sticht C, Gretz N, Truckenmüller R, Rouwkema J, van Blitterswijk CA: Sonic Hedgehog-activated engineered blood ves-

sels enhance bone tissue formation. Proceedings of the National Academy of Sciences. 2012;109(12):4413–4418.

[23] Correia C, Grayson WL, Park M, Hutton D, Zhou B, Guo XE, Niklason L, Sousa RA, Reis RL, Vunjak-Novakovic G: In vitro model of vascularized bone: synergizing vascular development and osteogenesis. PLoS One. 2011;6(12):e28352.

[24] Dong Z, Li B, Liu B, Bai S, Li G, Ding A, Zhao J, Liu Y: Platelet-rich plasma promotes angiogenesis of prefabricated vascularized bone graft. Journal of Oral and Maxillofacial Surgery. 2012;70(9):2191–2197.

[25] Behr B, Leucht P, Longaker MT, Quarto N: *Fgf-9* is required for angiogenesis and osteogenesis in long bone repair. PNAS. 2010;107:11853–11858. doi:10.1073/pnas.100331710.

[26] Zelzer E, McLean W, Ng YS, Fukai N, Reginato AM, Lovejoy S, D'Amore PA, Olsen BR: Skeletal defects in VEGF120/120 mice reveal multiple roles for VEGF in skeletogenesis. Development. 2002;129(8):1893–1904.

[27] Hill C, Jacobs B, Kennedy L, Rohde S, Zhou B, Baldwin S, Goudy S: Cranial neural crest deletion of VEGFa causes cleft palate with aberrant vascular and bone development. Cell and Tissue Research. 2015;361(3):711–722.

[28] Lui Y, Olsen BR: Distinct VEGF functions during bone development and homeostasis. Archivum Immunologiae et Therapiae Experimentalis. 2014;62:363–368.

[29] Zelzer E, Olsen BR: Multiple roles of vascular endothelial growth factor (VEGF) in skeletal development, growth, and repair. Current Topics in Developmental Biology. 2004;65:169–187.

[30] Yang Y, Tan Y, Wong R, Wenden A, Zhang L, Rabie ABM: The role of vascular endothelial growth factor in ossification. International Journal of Oral Science. 2012;4:64–68. doi:10.1038/ijos.2012.33